REMARKS

The Applicants thank the Examiner for carefully reviewing the present application and preparing the Office Action dated August 26, 2003. Claims 4, 6, 7, 15-20, and 25-74 are hereby cancelled without prejudice. Therefore, **Claims 1-3, 5, 8-14,** and **21-24** remain pending in the application. New claims 75-82 have been added. The Applicants respectfully submit that all pending rejections have been addressed and that this amendment places the pending claims in condition for allowance. Accordingly, the applicants respectfully request reconsideration and allowance of the pending claims.

Objections:

Claims 8 and 20 have been objected to because certain terms and phrases were underlined. In accordance with Examiner's instruction, these claims have been corrected in claim 8. As claim 20 has been cancelled, the objection is moot. These corrections do not affect the breadth of the claims are made only to correct certain typographical errors.

Claim 8 has been amended to more clearly claim the intended subject matter. The term "macromolecule" has been replaced by the term -- protein -- to remedy the antecedent basis issues presented by the pending claims.

Rejections Under 35 U.S.C. § 112, Second Paragraph:

Claims 1-6 and 13 stand rejected under 35 U.S.C. § 112, second paragraph as being indefinite. The Office Action has asserted that the term "smaller" is vague and indefinite. The Action has posited that the term smaller could mean could mean "smaller" due to three-dimensional volume or "smaller" due to having fewer residues. The Applicants agree with the Examiner, the fragmented parts could be either "smaller" due to three-dimensional volume or "smaller" due to having fewer residues, typically both. Either interpretation falls within the scope of the claims. Such a use is in accordance with the ordinary meaning of the word "smaller". Nevertheless, to expedite prosecution without narrowing claim scope, claim 1 has been amended to remove the term "smaller."

Additionally, **Claim 5** has been rejected under 35 U.S.C. § 112, second paragraph as being indefinite. The Office Action has asserted that the term "best fit" causes the claim to be vague because it is unclear what criteria are used to determine that a structure "best fits" the distance constraints. Distance constraints are described in many places in the specification. In one salient example, at page 9: lines 7-15 the "spatial constraints" are used as a defining distance criteria. Additionally, a detailed description of distance constraint determination is elaborated up in the Specification at 12:30-17:27 with another practical example being illustrated at page 30:lines 6-25. Models which best fit these constraints (as opposed to models which do not well fit the constraints) are selected. Such models do not necessarily exactly fit the constraints, they

merely provide better fits than some other models – as would be conveyed by conventional usage and understood by those of skill in the art.

It is respectfully asserted that the issues raised in items 9, 10, and 11 of the Office Action have been addressed and the terms at issue are well described in the Specification, and sufficiently clear such that one of ordinary skill in the art would not find such terms "vague". The Applicants respectfully request that the pending objections be withdrawn as to Claims 1-6 and 13 and that this claims be allowed to issue.

Rejections Under 35 U.S.C. § 112, First Paragraph:

Claims 1-6, 8-14, 20-25, and 74 stand rejected under 35 U.S.C. § 112, first paragraph as not reasonably providing enablement for the rejected claims. The Action acknowledges that the specification enables embodiments that employ cross-linking proteins. In order expedite prosecution, independent claims 1 and 8 have been amended to recite that the physical distance constraints comprise cross-links. Applicants wish to note that cross-links can take many different forms that link two or more non-contiguous residues or moieties by a chemical or physical constraint, covalent or otherwise. Applicants also note that the claimed invention, as presented in claims 1 and 8, embodies methods that make use of cross-link information in conjunction with other distance constraint information such as NMR data sets.

In view of the above, it is respectfully submitted that the enablement rejection under 35 U.S.C. § 112, first paragraph has been overcome. Withdrawal of the rejection is respectfully requested. All pending claims are believed to meet the requirements of § 112, first paragraph.

Rejections Under 35 U.S.C. § 102:

Claims 1-6, 8, 14, 20-23, 25 and 74 stand rejected under 35 U.S.C. § 102 as being anticipated by Lacroix, et al. (1997). Lacroix et al. describe a study of relative positions of some residues in γ-B monomer and the $(\gamma\text{-B})_2$ dimer found in the modular protease human C1. While the Lacroix et al. reference does describe use of cross-linking and fragmenting of this protein region, γ-B, followed by mass spectrometric analysis of the fragments and homology modeling to clarify the domain structure of the protein region, it does not describe or suggest various limitations of the claims as amended, particularly the analysis steps that make use of cross-link information for the protein under consideration. The invention of claims 1 and 8 requires selecting one or more candidate conformations for the protein under consideration by applying distance constraint information (obtained from the cross-link data) to the candidate conformations. In contrast, Lacroix et al. apply cross-link data to whole domains (of fixed conformation) to position those domains with respect to one another.

Relevant limitations of the claims at issue include the following:

A. providing a set of candidate three-dimensional conformations for the primary sequence of the protein; and

B. applying physical distance constraint information for the identified cross-link fragments to the candidate three-dimensional conformations to select one or more of said structures that best fit the distance constraint information.

These limitations have been added to the two independent claims, claims 1 and 8. While they did not appear as such in the claims as originally submitted, they are well supported by the instant specification. Limitation A is supported in the specification at page 27, lines 1-8, for example. Note that "threading" is one example of a range of such techniques; generally any technique that overlays the backbone of a protein under consideration on the backbone of a candidate protein structure. Limitation B finds support at page 27, lines 20-22, and pages 36-38, for example.

For context, the γ-B protein region described in the Lacroix et al. reference will now be discussed briefly. Initial recognition of a target by human C1 is mediated by the sub-unit designated C1q. Recognition by C1q triggers activation of a different sub-unit C1s-C1r-C1r-C1s by autolytic activation of C1r and then C1r-mediated conversion of C1s to the active enzyme responsible for proteolysis. Both C1r auto-activation and subsequent activation of C1s are mediated by its catalytic region, the $(\gamma\text{-B})_2$ dimer. $(\gamma\text{-B})_2$ is a non-covalent dimer that forms the core of the C1r-C1r dimer and the C1s-C1r-C1r-C1s tetramer.

Each γ-B monomer is found in the C-terminal region of the C1r sub-units. Each γ-B monomer is a single-chain polypeptide comprising a tandem repeat of so-called "complement repeat protein modules" (or CCP modules), a 15-residue intermediary segment, and a serine protease (B) domain. Activation occurs through cleavage of the Arg446-Ile447 bond, thereby splitting the molecule between the intermediary segment and the B domain and yielding two polypeptides (γ and B) connected by a single disulfide bridge.

Some early studies suggested that the γ-B monomers contain discrete domains. But apart from some preliminary data suggesting that each C1r γ-B monomer interacts with its neighbor in a "head to tail" configuration, no available information showed the structure and assembly of these regions. The authors of the Lacroix et al. reference sought to provide greater insight into the assembly of the domains of the γ-B monomer and the $(\gamma\text{-B})_2$ dimer by chemically cross-linking the $C1r(\gamma\text{-B})_2$ dimer and performing modeling studies.

The cross-linking produced intra-monomer cross-links (within a single γ-B) and inter-monomer cross-links (between the individual residues of the separate γ-B monomers in a $(\gamma\text{-B})_2$ dimer). The resulting cross-linked γ-B polypeptides were fragmented with a protease and subjected to mass spectrometry analyses. This analysis and subsequent sequencing of the fragments identified one intra-monomer cross-link between Lys426 of one CCP module and Asp688 of the serine protease B-domain. It also identified one inter-monomer cross-link between Gly280 of fragment γ and Glu493 of the B domain.

The cross-link information was used in conjunction with homology modeling to construct a three-dimensional model of the γ-B monomer, in which a CCP module interacts with a serine protease on a side opposite to both the active site and the Arg446-Ile447 activation site. Also, "a tentative three-dimensional model of the $(\gamma\text{-B})_2$ dimer was built, indicating a loose 'head to tail' association of the monomers, with the active sites facing opposite directions toward the outside of the dimer."

Thus, as pointed out in the specification of the present patent application, the Lacroix et al. reference (as well as the related Rossi et al. reference) showed that "domain-domain placement can be done" using intra-molecular cross-linking. See page 10, lines 8-14 of the instant specification. What these references do not suggest is use of cross-linking and subsequent analysis as claimed to actually produce a moderate resolution (e.g., about 3-5 Angstroms) three-dimensional model of the polypeptide. Lacroix et al. used two cross-links to merely propose, roughly, the relative positions of multiple "pre-built" domains of $(\gamma\text{-B})_2$ dimer. The claimed invention employs a combination of (i) providing a set of candidate three-dimensional conformations for the primary sequence of the protein, and (ii) applying physical distance constraint information for the identified cross-link fragments to the candidate three-dimensional conformations to select one or more of said structures that best fit the distance constraint information. See Figure 13, page 27, lines 1-22, and pages 35-38, for example.

Neither the Lacroix et al. reference nor the Rossi et al. reference suggests these steps. While they do discuss cross-linking polypeptide portions of the C1 complex, fragmenting the cross-linked product, and analyzing mass spectroscopy data of the fragments, they do not suggest analyzing the data in a manner that is relevant to the presently claimed invention. They use cross-link information to propose an assembly of relative large domains of the protein, each domain being pre-analyzed to define its conformation. They do not propose a set of candidate three-dimensional conformations (for the protein's primary sequence) and then select from this

set one or more structures that best fit the distance constraint information. Withdrawal of the art rejections is respectfully requested.

Applicants note that original claims 23 and 25 recite use of threading in the analysis. These claims, which recite one technique for providing a set of candidate conformations, were rejected under 35 USC §102 on the basis that homology modeling described in the Lacroix et al. reference "is similar to that of Rossi et al. 1995." The Rossi et al. reference describes a study of the γ-B component of the C1s module (as opposed to the C1r module under study in the Lacroix et al. reference). In the Office Action, the Rossi et al. reference is said to disclose "'threading' wherein a set of homologous three-dimensional structures is used as a reference template, sequences of proteins are aligned and the candidate structure is identified by comparing the said structure to the reference set." Citing Rossi et al. at page 7313.

It is respectfully submitted that, even if correct, this interpretation of Lacroix et al. (bootstrapping Rossi et al.) does not render the pending claims anticipated or obvious. The cited portion of Rossi et al. describes a program "O" to construct homology based models of various individual modules of the γ-B monomer and then assemble these using information provided by the chemical cross-linking. Note that the γ-B monomer is posited to have multiple separate modules as described above. Rossi et al. and Lacroix et al. used a homology modeling technique to separately characterize each of the modules (the B chain and two CCP modules). The cross-linking information was used to assemble these modules – not to "select one or more of said three-dimensional structures [from a set of candidate three-dimensional conformations] that best fit the distance constraint information" as claimed. See the discussion at pages 7318 and 7319 for an explanation of how Rossi et al. used cross-link information to locate and assemble previously defined modules into the overall γ-B structure. See also the discussion at page 6278 of the Lacroix et al. reference.

In short, nothing in Rossi et al. nor Lacroix et al. suggest using cross-link information (distance constraint information) in the manner claimed: "applying physical distance constraint information for the identified cross-link fragments to the candidate three-dimensional conformations to select one or more of said structures that best fit the distance constraint information."

Note also that limitations of various dependent claims are not suggested by the cited references. For example, claim 22 requires "constructing a virtual library of proteolyzed products which library is indexed by a criteria selected from the group consisting of monoisotopic data and average mass data." And claim 25 requires "applying physical distance

constraint information for the identified cross-link fragments is performed with the use of an equation" as shown. Despite assertions to the contrary in the Office Action, the cited references nowhere suggest or imply either of these limitations.

In view of the foregoing amendments and discussion, it is respectfully submitted that Lacroix et al. does not anticipate independent claim 1 or independent claim 8. Withdrawal of the rejections under §102 is respectfully requested. Dependent Claims 2, 3, 5, 14, and 21-23 are also believed to be in condition for allowance. Claims 4, 6, 7, 20 and 73 are cancelled without prejudice and so the grounds for rejection of theses claims are now moot.

Rejections Under 35 U.S.C. § 103:

Claims 9-13 are rejected as being unpatentable over Lacroix in view of Mitra. First, Claim 8 (upon which Claims 9-13 all depend) overcomes the Lacroix reference as explained above. Thus, for at least those reasons the Lacroix reference does not teach all of the limitations of the underlying base claim (Claim 8). Consequently, any rejection of Claim 8 or its dependent claims under § 103 must, at a minimum, establish a prima facie case of obviousness with respect to all the limitations of claim 8. Mitra does nothing to remedy the deficiencies of Lacroix (e.g., neither Lacroix or Mitra teach the Claim 8 limitation of "applying physical distance constraint information for the identified cross-link fragments to the candidate three-dimensional conformations to select one or more of said structures that best fit the distance constraint information"). So for at least the foregoing reasons, it is respectfully submitted that the combination of Lacroix and Mitra are insufficient to establish a prima facie case of obviousness. Withdrawal of the rejections under § 103 is respectfully requested.

Added Claims

Claims 75 and 76 have been added to the pending claim set. These claims depend from amended claim 1or 8, both believed allowable as amended. Consequently, for at least the reasons set forth above with respect to Claims 1 and 8, it is submitted that Claims 75 and 76 are in condition for allowance. Moreover, Claim 75 includes the further limitation of "assessing said conformations' compatibility with computed physical properties for the conformations." Claim 76 specifies that the assessing "includes using at least one technique selected from among: calculating the distribution of hydrophobic/hydrophilic amino acids; mapping a hydrogen-bond network; locating disulfide bridges; functional mapping of mutagenesis data; assessing the complementarity of the hypothetical structure's secondary structure and the secondary structures predicted for the sequence; insuring that critical electrostatic interactions are preserved;

identifying sites of van der Waals clashes; and evaluating the sequence-structure-sequence similarity.." These added limitations find support in the Specification, for example, at page 24; lines 23-34 and elsewhere. The new limitations are not taught in the cited references, therefore it is respectfully submitted that claims 75 and 76 are in condition for allowance.

Of interest, new claim 78 recites "cross-linking residues of the protein such that the number of cross-links in the protein is at least about 10% of the number of amino acid residues in the protein." This limitation finds support at, for example, the bottom of page 12 to top of page 13 and page 25, lines 15-19. The Lacroix et al. reference employs only two cross-links total, a number many times lower than that required by claim 78. This is not surprising since Lacroix et al. were merely interested in using cross-link information to roughly position pre-solved domains with respect to one another. Using a comparatively large number of cross-links as claimed provides sufficient information to truly select an appropriate three-dimensional conformation of moderate resolution. It is respectfully submitted that claim 78 and its dependent claims 79-82 are patentable over the art of record.

New claim 77 recites a method of claim 1 or 8, "wherein the three-dimensional structural information comprises a three-dimensional structure of the macromolecule having a resolution of about 2-5 Angstroms." Support for this new claim is found at page 29, lines 19-21 and elsewhere. New claim 79 provides a similar limitation with regard to claim 78. It is respectfully submitted that these claims are patentable for at least the same reasons advanced above for claims 1, 8, and 78.

Applicants respectfully submit that all pending claims are allowable and respectfully requests a Notice of Allowance for this application from the Examiner. If the Examiner wishes to telephone the applicants representative concerning any matter pertaining to this case, the Examiner is cordially invited to do so at the telephone number set out below. The Commissioner is hereby authorized to charge any additional fees to Deposit Account 500388 (Order No. UCSFP001).

Respectfully submitted,
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